Supplementary Materials

aeBlue Chromoprotein Color is Temperature Dependent

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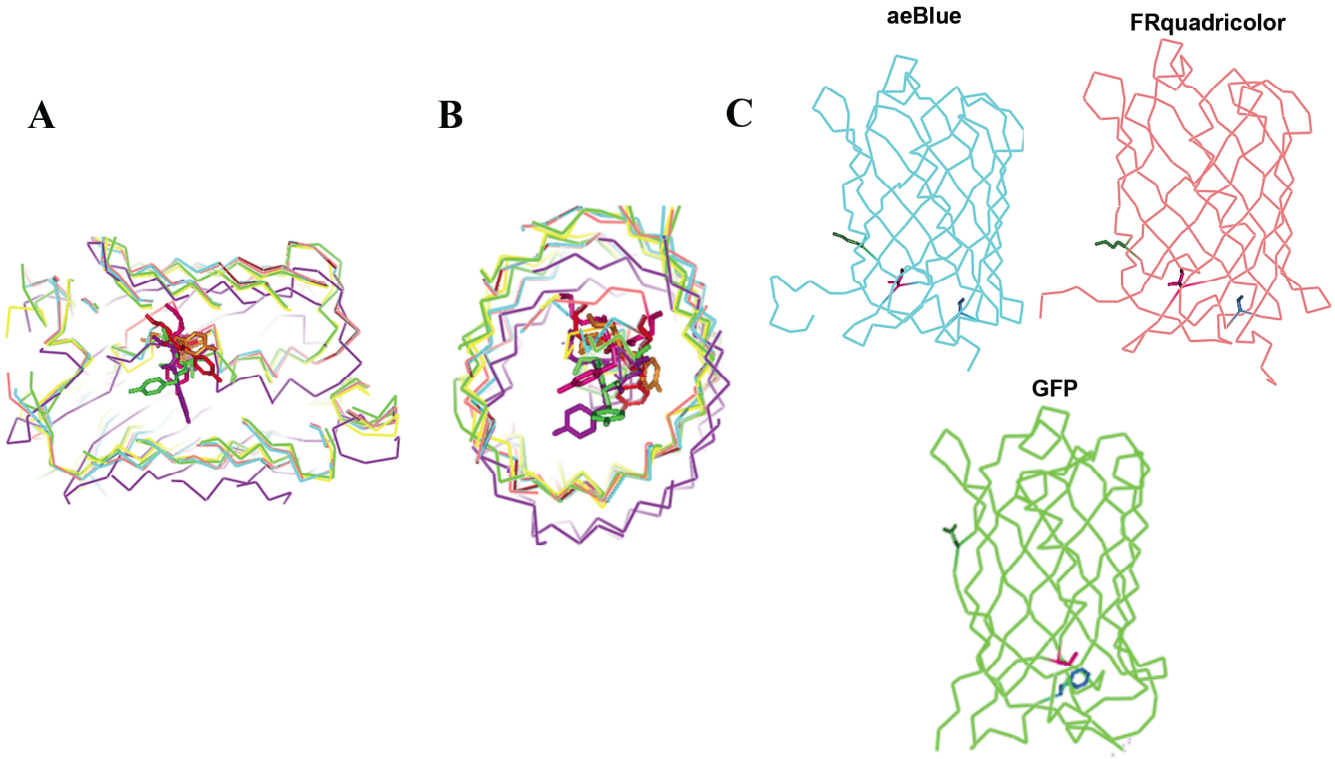

Figure S1. Structural alignment of the chromoproteins aeBlue, AmilCP, GFP, the Fast-FT blue fluorescent protein and FR quadricolor (Fluorescent timer). Panel **A** and Panel **B** show the residues needed for chromophore formation for each protein. Panel **A** side view and Panel **B** top view. Color identification: aeBlue in red, AmilCP in orange, GFP in green, Fast-FT in light pink, and FRquadricolor (timer) in hot pink. In panel **C** we show the residues needed for color conversion if FRquadricolor and the location in aeBlue and GFP. Residue 70 in hot pink, 83 in sky blue and 146 in forest, GFP numbering. Color names according to PyMol.

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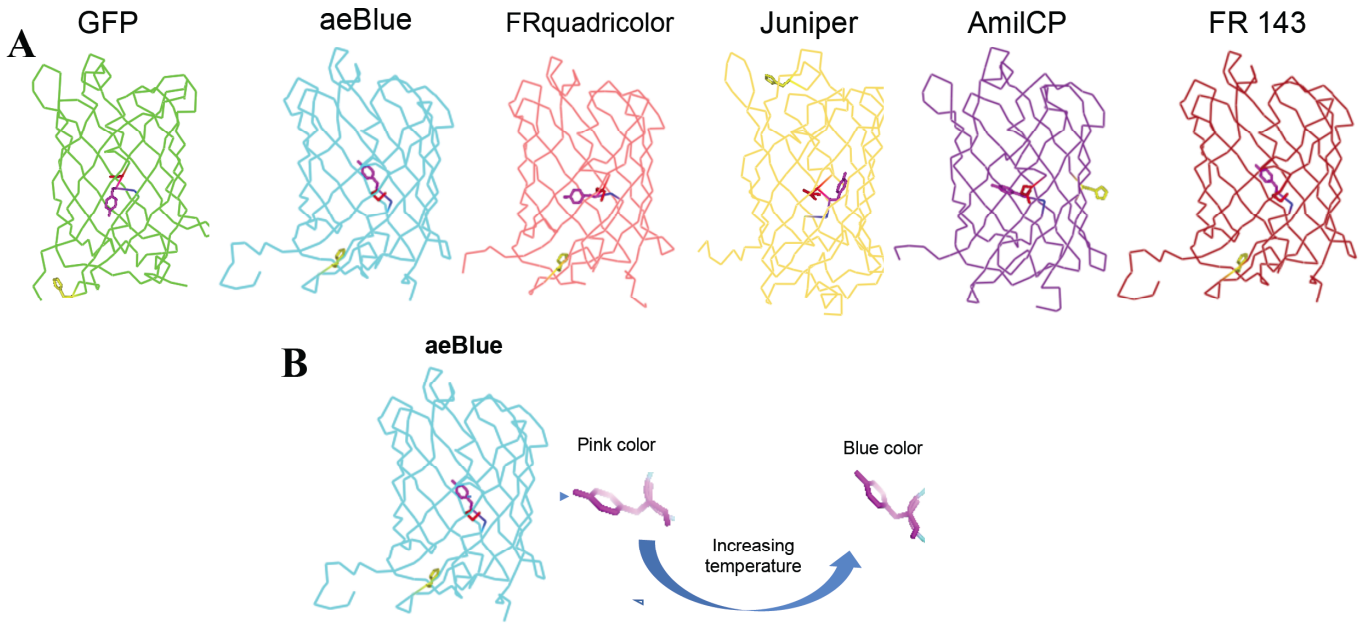

Figure S2. Panel **A**, Chromophore aromatic residue comparison between GFP, aeBlue, Juniper, AmilCP, Fast-FT and FR quadricolor proteins, GFP numbering. Residues 65 in red, 66 in magenta (aromatic), 67 in blue and the conserved histidine in yellow. The position of the aromatic residue is in accordance with the GFP sequence. GFP (PDB:2AWK), aeBlue, Juniper, AmilCP (Accession number: AY646075), Fast-FT and FR quadricolor (PDB:1UIS). Color names according to PyMol. Panel **B**, proposed model for color shift in aeBlue. The aromatic position but not the quaternary structure is shifted in the protein, rendering a position similar to the red-color proteins, such as the FRquadricolor.

Table S1. Oligonucleotides used in this study.

Primer name	5' to 3' sequence	Application
aeBlue forward	CCCGGATCCATGGCGTCACTTGTAAAAAAAGA	The primer pair used to amplify the aeBlue sequence from pIJ201 plasmid and clone it into pQE30 using BamHI and HindIII restriction sites (underlined)
aeBlue reverse	CCCAAGCTTTTATCAATGATGCCCCAACTT	
pQE30 promoter primer	CCCGAAAAGTGCCACCTG	Primer pair to amplify and verify aeBlue sequence in pQE30 plasmid
pQE30 reverse primer	GGTCATTACTGGAGTCTTG	

Supplementary Video S1. aeBlue-pink protein was filmed in a water bath with continuous heating. The temperature was monitored with a surface thermometer. The measurement was conducted using the beaker filled with water at 4 °C and gradually heated up to 60 °C. Color changed occurred 20 min after heating started. The homogeneity of the beaker temperature was maintained by gentle stirring. The presented material is shown three photograms per second.